THE LIFE-CYCLE OF THEILERIA PARVA — THE CAUSE OF EAST COAST FEVER IN CATTLE IN SOUTH AFRICA.

A GENERAL REVIEW.

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In a previous communication* I reported on the developmental stages of *Theileria parva* (*Piroplasma parvum, Babesia parva*) in the organs of cattle, and demonstrated the various stages, and I am now able to explain practically the whole life-cycle of this parasite. I then mentioned (1) that *Theileria parva* (as I call the parasite of East Coast fever), with Bettencourt, Franca, and Borges, cannot be identified with *Babesia mutans* (*Piroplasma mutans*); and (2) that the forms found in the organs of cattle represent a specific stage in its life-cycle, the forms of which stage are of great importance from a diagnostic point of view. Recent investigations have completely corroborated my previous statements.

I divided the development of the parasite of East Coast fever in the organs into two generations—distinguishable by their morphology—i.e. agamogonous and gamogonous. As indicated by the name, the former signifies certain forms which multiply agametically, that is to say, forms which are not capable of performing a sexual function. It is only after the elimination of nuclear substance (reduction of nucleus) that parasites result from these agamogonous stages which develop into the gamogonous generation. The gamogonous generation then supplies sexual forms which copulate when they obtain access to the stomach of the transmitting host.

It is generally known that every protozoon undergoes a process of fertilization in its life-cycle; this fertilization acts on the organism as a regulating mechanism. The propagation or multiplication of the protozoon is not of necessity connected with fertilization; it may precede or succeed it. In the course of the phylogenetic evolution of parasites, and especially of the blood protozoa, a definite alternation of generations has developed. The original host is undoubtedly the transmitter (Ektoparasite) in which copulation and the succeeding encystment or an agamagonous development takes place. In the intermediate host (man or animal) the further agametic multiplication proceeds, ending in the progamogonous or gamogonous generation, that is to say, with the formation of the proper sexual individuals. The latter are only able to undergo further development in the transmitting agent.

1

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In addition to this typical fertilization (the copulation of male and female cells), we know of another form, namely, parthenogenesis, where the nucleus undergoes a process of self-fertilization. This phenomenon is extremely important for the explanation of relapses in protozoan diseases. It also plays an important rôle in the question of immunity. In protozoan diseases, in which parthenogenesis of the corresponding parasites occurs, we rarely if at all meet with an absolute immunity. It is a relative immunity designated *immunitas non sterilisans.* If we meet with a protozoan disease where the female form (macrogametocyte) cannot undergo parthenogenesis, we know that in most cases as soon as the man or animal recovers, a complete immunity is effected.

I have given this general review for the better understanding of the life-cycle of *Theileria parva*, and as will be seen later, many facts in that cycle, and many conditions of the disease itself, can be explained by the biology and physiology of the protozoon.

In South Africa, the tick which is of chief importance and with which I have experimented exclusively in the latter part of my investigations is *Rhipicephalus appendiculatus. Rh. evertsi*, which is also a transmitter of East Coast fever, was only used at the commencement of my investigations.

It is well known that the parasite of East Coast fever does not pass through the egg, but the tick can only transmit the diseases in either the nymphal or imago stage. For the completion of its cycle the tick requires three changes of host. If a larval tick attaches itself on an animal suffering from East Coast fever, it leaves the host as soon as it is replete, the length of time it remains on the host depending chiefly on the external temperature, a fact which is the cause of many difficulties in the study of the East Coast fever parasite in the tick itself. After having dropped off the animal, the larval tick moults sooner or later, according to external favourable or unfavourable climatic conditions. Warmth undoubtedly influences the rapid development of ticks. It is only after the tick has moulted into the nymphal stage that it seeks a new host, where it again becomes repleted. It leaves this second host to moult again for the second time in order to finally arrive at the imago stage on the third host. It leaves this third host either as a male, which has no other task than to mature and to fertilize the female, or as a female to become fertilized and to replete itself with a great quantity of blood necessary for the formation of eggs. If the tick has been infected as a larva, it can only transmit the disease in the nymphal stage; if it has become infected as a nympha, it can only transmit the disease as an imago.

An infected tick purifies itself completely from all infection once it has bitten an animal. If infected as a larva it can only become re-infected as a nympha, but never as an adult tick. It only transmits the disease in the last stage if it has infected itself in the previous stage as a nympha. Its purification can be effected by biting on any mammal; an ox is not exclusively necessary. As far as is known, *Theileria parva* is only pathogenic for cattle. The biological peculiarity in the cleansing of the ticks from infection has been made use of in South Africa for the combating of East Coast fever.

A reference to the plate will help to explain the whole life-cycle of *Theileria parva*, and the more important forms of the parasite as seen in the microscope are shown.

With the bite of an infected tick, small uninuclear forms arrive in the blood circulation of cattle, and undergo further development in the organs, more especially in the lymphatic and haemo-lymphatic glands, in the bone marrow, and in the spleen (figure 1). These small parasites represent the sporozites or, according to Hartman's nomenclature, the agametes of the first or metagametic generation. They are only found after the ticks have moulted, that is, at the time when the tick is ready to seek a new host. I have not yet been able to trace these forms in cattle, and I have not found them in clean ticks (not infected), control ticks, or in ticks which have become purified by biting.

If we carry out a systematic puncture of the glands and an occasional puncture of the spleen, commencing on the first day of the disease—that is to say from the day on which the tick attaches itself—we are able to trace the further development of the parasite. The superficial cervical and precrural glands are the most convenient for puncturing purposes. The first forms of the parasites are occasionally found on the twelfth or thirteenth days, but it is difficult to state the exact date, as the incubation time varies in the different experiments. These parasites reach the size of about 0·8 to 1 micron, and at first are found free. During the following days they are seen intracellularly, i.e. in the large lymphocytes, and very rarely in other leucocytes. They grow in size rapidly within the next few days (figures 2, 7*a*, and 7*b*). These agametes increase in size, and after multiplication of the nuclei grow into agamonts which finally split up into as many segments as there are nuclei. On an average the agamont measures about 10–12 microns, rarely 12–15 microns.

Naturally the larger ones contain the greater number of nuclei. In the intracellular forms a considerable number of segments, agametes of the second generation, are frequently found, caused by double infection. As a result of this parasitism, the lymphocyte is destroyed and accordingly it is not surprising to meet irregularly formed agametes if the infected lymphocyte dies off before the agamont has divided into its daughter forms.

Division of the nuclei takes place by amitosis; in exceptional cases we meet indications of primitive mitosis in moist fixed smears or sections. The number of nuclei increases very considerably by successive fission processes, and may amount to more than forty or fifty. The form of the nuclei and their structure is characteristic for the process of agamogony. The nuclei do not possess an envelope, they have an irregular rugged form and no compact structure. During life they do not appear very refractile, they do not show much affinity for the various stains. When stained with haemotoxylin or giemsa, fixed and treated by the moist method, the stain easily escapes from the nuclei. The development of the agametes from the youngest to the full-grown agamonts (figure 2*a* and *b*, figure 6*a* and *b*) and to succeeding schizogony into agametes (merosoites) can repeat itself.

It must be stated that in the animal the disease commences with the appearance of these agamogonous forms. The temperature begins to rise, and reaches the maximum with the formation of the gamogonous forms; the agamont which does not produce any more agametes divides into gamonts (figure 9*a*) after its nuclei have eliminated the vegetative substance (figures 8*a* and 8*c*) by the formation of chromidia, and the process of reduction.

These gamonts increase both in size and in numbers of nuclei, and finally divide up into gamotocytes which invade the red corpuscles, and now represent the parasite of East Coast fever known under the name of *Theileria parva*.

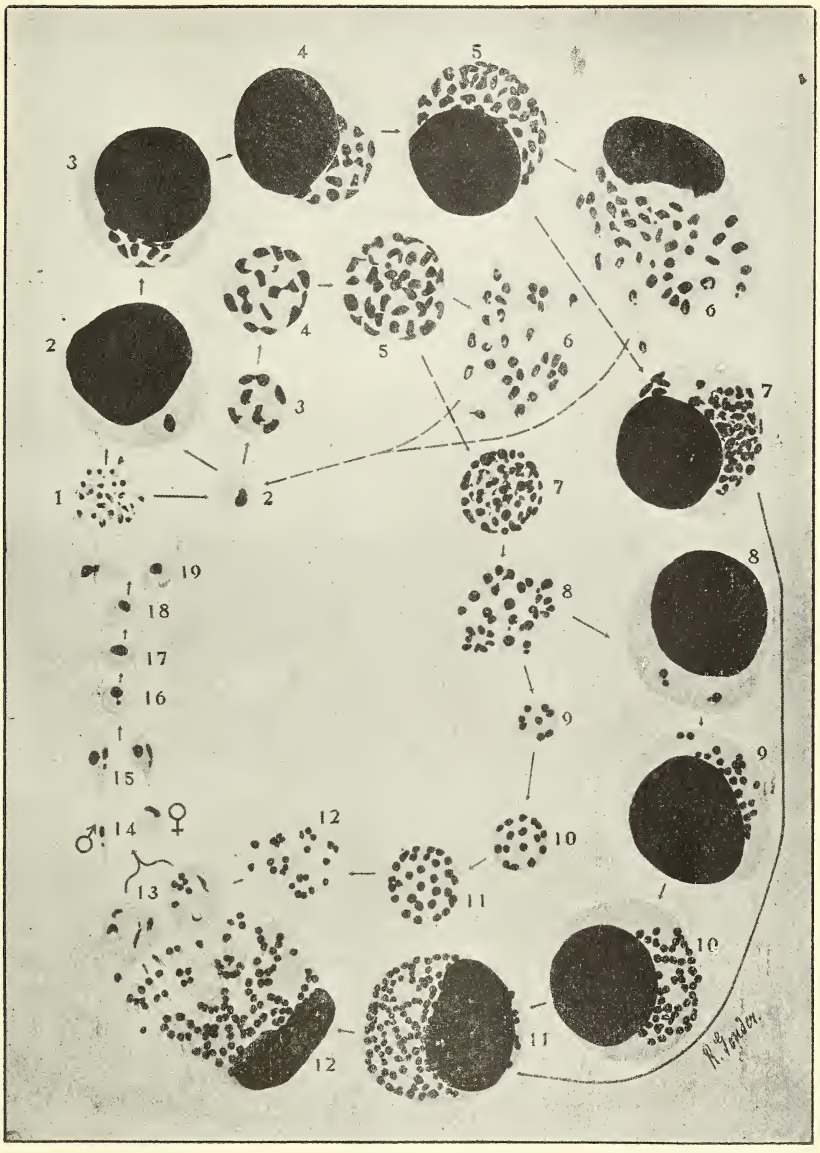
The gamogonous forms are clearly distinguishable from the agamogonous form by their nuclei. The youngest gamonts (figures 9*b* and 10*c* and *b*) measures about 0·8 micron, and possess a strongly refractile nucleus which takes the various stains intensively. In advanced stages (figures 11-13*a* and *b*) the nuclei possess distinct karyosomes, and occasionally in the youngest forms, along with the main nucleus, a second smaller nucleus is found, which may be compared with the blepharoplast of the flagellum, and which is of importance for the systematic position of our parasites.

The multiplication of the nuclei in the gamonts takes place by a primitive mitosis in such a way that the karyosome splits the two fragments of the nucleus. Finally the gamont divides into gametocytes (figure 14*a* and *b*) after leaving a residual body staining blue with giemsa. As already stated, the nuclei are characterized during life by strong refraction, and accordingly are easily distinguishable from the granules of the lymphocytes, although contrary to what is seen in the agamogonous forms, their shape is more regular, being almost oval.

Usually the intracellular gamonts supply a far greater number of gametocytes than those that are free. This is partly due to double infections similar to what is found in intracellular agamogonous forms. The schizogony of the reduced agamonts within the lymphocytes (figure 8*b*) and the further development of the young gamonts may take place at the same time, so that naturally there are a great number of gametocytes present. In many cases I could count 150–200 gametocytes, the products of the gamonts in one single lymphocyte.

The evolution forms of *Theileria parva* of the gamogonous and agamogonous stages as described above have been known for some time under the name of Koch's bodies or plasma bodies. They have been the subject of much discussion, especially after Martin Meyer believed he had found similar bodies in other diseases, including piroplasmosis. As I personally have seen preparations of Martin Meyer, and not having been able up to the present in a single case of piroplasmosis or other disease of a protozoan origin, to trace forms which could be mistaken for cycle forms, I must say that the so-called reaction products, as designated by Martin Meyer, have nothing to do with the so-called Koch's bodies.

I have been able to follow up the segmentation during life of the gamonts into gametocytes, and to demonstrate these and other stages both in cattle and ticks in their natural state. With the large material



THE LIFE-CYCLE OF THEILERIA PARVA.

placed at my disposal at the Government Veterinary Bacteriological Laboratory, for protozoological studies, amounting to about 80–100 head of cattle used in East Coast fever experiments, I frequently had the opportunity of controlling my observations.

In the intracellular forms it occasionally happens that through the dissolution of the lymphocyte, these escape and invade the blood corpuscles as gamonts. It also sometimes occurs that very small free gamonts are seen in the lymphatic glands, etc., which, without reaching any great size, divide into gametocytes. Such small gamonts can finally further divide into gametocytes in the blood corpuscles, but I very rarely succeeded in tracing their development in the blood. Mistakes can easily occur, since the parasite very rapidly leaves the corpuscles when the blood is brought into different physiological conditions—a fact which can also be noted under the microscope. The formation of " cross-forms " as a rule takes place in the organs.

The gametocytes in the blood are sufficiently well known, as is also the rapid increase of their numbers. With such a heavy infection of the blood, one is struck with the fact that no pathological lesions are found in the red corpuscles, and only towards the end of the disease may a slight anisocytosis be observed. If a further development, or rather if an increase would take place in the blood, one would expect to find changes in the red corpuscles such as are found with the malaria-plasmodia of apes, parasites found in bats, *Babesia mutans*, etc., either due to the liberation of the toxines, or by the simple mechanical influences caused by immigration and emigration of parasites into and out of the corpuscles. In the stages of the East Coast fever parasite found in the blood, we do not notice such changes of the corpuscles, and the absence of such changes can be explained by the life-cycle as explained before. With the formation of the gametocytes, the cycle of *Theileria parva* in the animal arrives at a definite conclusion—the animal either recovers or dies.

In cattle which recover from the disease, a general decrease of the parasites is noted in the blood after the crisis. The agamogonous forms disappear, and with this the fever gradually subsides. The gamogonous stages do not develop beyond the formation of gametocytes, these are the endoglobular parasites. Since we do not find parthenogenesis, the animal is completely protected against relapses, and recovery leaves a complete sterile immunity. No tick can infect itself on this animal and no infected tick can infect such a beast.

In my studies I also had an opportunity of seeing cases ending rapidly with death (acute forms of East Coast fever), in which I could not detect any parasites in the blood or gamogonous forms in the glands, but only the agamogonous stages. Therefore I am of opinion that in the first instance agamogonous forms are those which cause the disease, or which, in other words, possess the toxines. In some of the experiments of Doctor Theiler, undertaken for the purpose of immunizing cattle, I have noticed agamogonous stages in the blood after inoculation, and it may be expected that these animals will prove immune to the disease.

The transmission of the disease with blood has hitherto not been successful, and this may be explained by the fact that the gametocytes do not develop any further, and do not undergo parthenogenesis. If it is possible to transmit the disease with blood, such can certainly only be the case at the beginning before gamogonous forms appear. In the transmission experiments with organs, as undertaken at the Laboratory, agamonts are inoculated, that is, forms which are capable of further development.

The parasite in the red corpuscle can only undergo further development after it has entered into the tick. The gametocytes contained in the blood then develop further. When copulation occurs in the tick, micro- and macrogametocytes can be distinguished in the blood. The gametocyte, which is ring-shaped or pear-shaped, takes either on the elongated or so-called bacillary form, or it grows into a broad ring or becomes more pear-shaped. The former represents the microgametocytes, the latter the macrogametocytes. (Figures 16 and 17.)

After the infected blood corpuscles have reached the stomach of the tick, the parasites emigrate within the first half-hour. A great number perish. Only the mature gametocytes grow into gametes and mix with each other. The microgametes contain a distinct small nucleus similar to the centrosome or blepharoplast (Browazek and Hartman) of other organisms, which acts as the initial agency for the development.

The fertilized macrogamete (figure 18) " rounds off " after a karyomyoxis and from this the ookinete results and similar to other blood parasites takes the shape of a retort changing into that of a gregarine, and finally grows into the elongated ookinete. The ookinetes can be recognized by their activity—they double back and stretch out rapidly, and show contractile movements like gregarines.

The forms given in figures 19–22, I have as yet found only in infected ticks on the eve of moulting. During the moulting process, I was unable to trace any intermediate forms which would lead from the ookinetes to the agametes of the first generation (sporozoites) as shown in figure 1. With the formation of the agametes the evolution of *Theileria parva* is complete, and when these agametes find their way into a beast the described cycle commences afresh.

Recently some publications have been made by Nuttall, Fantham, and Porter on *Theileria parva*, in which the evolution cycle of the East Coast fever parasite has been studied in stained dry preparations. Since these investigators did not mention in any way the evolution forms in the organs, and their investigations have not come to a conclusion except in regard to the already known facts, such as number and shape of the blood parasites, I cannot enter into any discussion for the time being.

I only wish to repeat that my observations have been made on living material, and with preparations fixed by moist methods, and I wish to draw attention to the detailed illustrated report due to appear shortly in the " Archiv. fur Protistenkunde ", and in the report of the Government Veterinary Bacteriologist of the Transvaal, in which I have particularly referred to literature on the subject.

EXPLANATION OF THE PLATE.

Figure 1 .. .. .. Agametes of the first generation (metagametes).
Figure 2, *a* and *b* .. Agamont with one nucleus.
Figure 3, *a* and *b* .. Agamonts with several nuclei.
Figure 4, *a* and *b* .. Medium-sized agamonts.
Figure 5, *a* and *b* .. Large agamonts with numerous nuclei.
Figure 6, *a* and *b* .. Agamonts undergoing schizogonie.
Figure 7, *a* and *b* .. Agametes.
Figure 8, *a* and *b* .. Reduction forms of agamonts.
Figure 9, *a* and *b* .. Segmentation of reduction forms of agamonts.
Figure 10, *a*, *u*, and *b* .. Young agamonts.
Figure 11, *a*, *u*, and *b* .. Medium sized agamonts with several nuclei.
Figures 12–13, *a*, *u*, and *b* .. Large agamonts with numerous nuclei.
Figure 14, *a*, *u*, and *b* .. Agamonts undergoing schizogonie.
Figure 15, *a*, *u*, and *b* .. Free gametocytes.
Figure 16, *a*, *u*, and *b* .. Gametocytes in the red blood corpuscles.
Figure 17, *a*, *u*, and *b* .. Micro- and macrogametes in the stomach of the tick.
Figure 18, *a*, *u*, and *b* .. Copulation.
Figure 19, *a*, *u*, and *b* .. Karyomyxis.
Figures 20–21, *a*, *u*, and *b* .. Formation of the ookinetes.
Figure 21, *a*, *u*, and *b* .. Retort forms of ookinete.
Figure 22 .. .. .. Ookinete.